

In the Claims:

Please cancel claims 6 and 12-27 without disclaimer or prejudice to the inclusion of the subject matter contained therein in any later filed continuation or divisional application(s).

Please amend claims 1-5, 7-11, and 28-29, and add claims 30-35 as set forth below.

1. (Currently amended) A method for producing therapeutic human T regulatory cells (Treg cells) with enhanced ~~suppressive~~ suppressor activity, said method comprising:

selecting a sample of human CD4⁺ T cells; and

contacting said sample with an anti-CD25 antibody;

isolating cells that bind to said anti-CD25 antibody from said sample using a population of human CD4⁺CD25⁺ suppressor T cells using a lower titer of anti-CD25 in a modified double column magnetic antibody cell sorting (MACS) purification procedure comprising a double column purification procedure, to produce an isolated population of human CD4⁺CD25⁺ Treg cells; and

~~ex vivo, long term, culture-expanding~~ [[the]] said population of human CD4⁺CD25⁺ Treg cells by GMP-approved methods, wherein said culture-expanding comprises contacting said isolated population of human CD4⁺CD25⁺ Treg cells with immobilized anti-CD3 antibody and immobilized anti-CD28 antibody at a predetermined ratio, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is less than 1, thereby activating potent long term producing therapeutic human Treg cells with enhanced suppressor activity in the isolated, culture-expanded cells, wherein prior to expansion the natural population of CD4⁺CD25⁺ suppressor cells represents a low percentage of the total isolated CD4⁺ T-cell population.

2. (Currently amended) The method of claim ~~[[1]]~~ 30, wherein ~~isolation of the CD4⁺CD25⁺ cells~~ said isolation step comprises a high level of stringency.

3. (Currently amended) The method of claim 2, wherein ~~said isolation of the CD4⁺CD25⁺ cells~~ isolation step further comprises ~~purifying the isolate by~~ substantially

enhancing CD4⁺CD25^{bright} cells in ~~[[the]]~~ said isolated population, while substantially depleting CD25^{dim} cells in ~~[[the]]~~ said isolated population.

4. (Currently amended) The method of claim 3, wherein said ~~purification method~~ isolating step comprises contacting the ~~isolate~~ selected human CD4⁺ T cells with 2 μ l of said conjugated anti-CD25 magnetic microbeads at a predetermined bead/cell ratio per 10⁷ total cells, and wherein the double column purification procedure comprises purifying by running the bead/cell composition over a magnetic column to separate bead-bound cells, washing, and re-eluting over a second magnetic column, and again washing until <1-2% of nonsuppressor cells remain in the purified isolate.

5. (Currently amended) The method of claim 1, wherein said culture-expanding step produces the CD4⁺CD25⁺ cells ~~comprises activating the isolated CD4⁺CD25⁺ cells with a cleavable cell-sized, antibody-coated, magnetic microbeads, thereby amplifying the culture-expanded Treg-suppressor cells over a sufficient period of time until there exists in the cell culture an effective amount of suppressor cells to achieve therapeutic suppression of an immune or autoimmune response in a human.~~

6. (Canceled)

7. (Currently amended) The method of claim ~~[[6]]~~ 1, wherein said culture-expanding step further comprising supplementing media for culture-expanding the cells comprises contacting said isolated population of human CD4⁺CD25⁺ Treg cells with IL-2.

8. (Currently amended) The method of claim ~~[[6]]~~ 1, wherein said isolated population of human CD4⁺CD25⁺ Treg cells are expanded further comprising achieving at least 10-20 fold expansion of the cells within in 14 days of culture in said culture-expanding step.

9. (Currently amended) The method of claim 8, wherein said isolated population of human CD4⁺CD25⁺ Treg cells are expanded further comprising achieving at least 100-fold expansion of the cells by culturing the cells for an additional 1-2 weeks.

10. (Currently amended) The method of claim [[6]] 1, further comprising generating ~~suppressor~~ therapeutic human Treg cell lines that retain long term down-regulatory suppressor function.

11. (Currently amended) The method of claim 1, wherein the sample of human CD4+ T cells is selected from the group consisting of whole or partially purified blood or hematopoietic cells, selected from the group consisting of peripheral blood mononuclear cells, peripheral blood lymphocytes, spleen cells, tumor-infiltrating lymphocytes and lymph node cells, and bone marrow and peripheral bone marrow cells.

12-27. (Canceled)

28. (Currently amended) The method of claim [[27]] 1, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is at least 1:5.

29. (Currently amended) The method of claim 10, wherein ~~the cells~~ said therapeutic human Treg cell lines retain long term down-regulatory suppressor function for at least three weeks.

30. (New) The method of claim 1, wherein said anti-CD25 antibody is directly conjugated to a magnetic microbead.

31. (New) The method of claim 1, wherein said MACS purification procedure is an indirect method, wherein said isolating step further comprises contacting said sample to magnetic microbeads conjugated to a secondary agent that binds to said anti-CD25 antibody.

32. (New) The method of claim 31, wherein said isolating step comprises a high level of stringency.

33. (New) The method of claim 32, wherein said isolating step further comprises substantially enhancing $CD4^{+}CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population.

34. (New) The method of claim 33, wherein said isolating step comprises contacting the selected, anti-CD25 antibody-contacted human $CD4^{+}$ T cells with $2\mu l$ of said magnetic microbeads per 10^7 total cells, and wherein the double column purification procedure comprises purifying by running the bead/cell composition over a magnetic column to separate bead-bound cells, washing, and re-eluting over a second magnetic column, and again washing until $<1-2\%$ of nonsuppressor cells remain in the purified isolate.

35. (New) The method of claim 31, wherein said anti-CD25 antibody is conjugated to FITC and said secondary agent is an anti-FITC antibody.